Table 3: Variant lovE Mutations

lovE allele	lovFp-neo Mediated G418R	MO oligos used for random PCR mutagenesis	Amino Acid Change 1	Amino Acid Change 2	Amino Acid Change 3	Amino Acid Change 4	Amino Acid Change 5	Amino Acid Change 6	Amino Acid Change 7	Amino Acid Change 8	Amino Acid Change 9	Amino Acid Change 10	Amino Acid Change 11
1	-/+	2624/2654	H253R	S341P									
2	+/-	2624/2654	R121W	S133L	S322G								
3	+++	2624/2654	C73R	A83V	T135I								
4	++	2624/2654	C73R	E177G									
5	++	2624/2654	C73R										
6	+/-	2624/2654	C153Y	E197K	T281A								
7	+	2624/2654	C73R	T256A	N466S								
8	+++	2624/2654	C73R	E141V									
9	++	2624/2654	C73R	E303K									
10	+++	2624/2654	Q41K										
16	+++	2680/2686	Q41K	P16A	G23S	T9M	Q362E						
19	+/-	2700/2701	R21H	S34A	Q80H	A84S	E303D	H374D	A440T	A441V	C445S	P469S	
20	+	2700/2701	F31L	T409I									
21	+++	2700/2701	F31L	M97I	E113D	D146N	P163S	N367I	H458Y				
30	+/-	2681/2686	I43V	Q295L									
31	++	2680/2686	F31L	P101S	C153R	C159S	E162K	R293L	S311N				
32	++	2680/2686	L14I	E18V	G138C	E338G	V361L	P389S	N400S				
33	++	2680/2686	Q41R	S174Y	A402T								
34	++	2680/2686	F31L	T52I	P101Q	P108S	V111I						
36	+/-	2700/2701	D85N	I143F	M232I	T315I	S382Y	M385K					
37	++	2700/2701	T46I	Q62R	K77R	S323C	N367Y	V373I					
38	-/+	2700/2701	Q41R	T294I	P310L	G337D	P389L	A394V	G436S				
39	+	2680/2686	T52N	V111I	T139	V184I	T281A						
40	+++	2680/2686	Q41R	D4E	V87I	D110E	E141K	A189T	N276D	T347R	N367I	Q377R	A425T
41	-/+	2680/2686	D131N	S133L	R312G	A429G							
wild-type	-	N/A	N/A										

Table 4 summarizes amino acid substitutions that were isolated multiple times, suggesting that they are particularly important for improving *lovE* variant activity on *lovF*p-*HIS3*p-*neo* expression.

Table 4: *lovE* Mutations Isolated Multiple Times

Amino Acid Change	Number of Times Isolated in *lovE* 1-41	*lovE variant*
F31L	4	20, 21, 31, 34
Q41K	2*	10, 16
Q41R	3*	33, 38, 40
T52I/T52N	1 each	34, 39
C73R	6*	3, 4, 5, 7, 8, 9
P101S/P101Q	1 each	31, 34
V111I	2	34, 39
S133L	2	2, 41
E141V, E141K	1 each	8, 40
C153Y/C153R	1 each	6, 31
T281A	2	6, 39
N367I/N367Y	2/1	21, 40, 37
P389S/P389L	1 each	32, 38

* allele was isolated in additional *lovE* variants that were not fully sequenced

Example 5: Increased *lovF-lacZ* Expression in *S. cerevisiae*

In order to quantify the increase in *lovF* expression, β-galactosidase activity was measured in *lovE* variant transformed *S. cerevisiae* strains that also harbored *lovF*p-lacZ reporter derivative plasmids. *lovF-lacZ* reporter derivative plasmids were constructed as follows.

Plasmid MB1918 contains the *lovF*p-*lacZ* reporter gene. It can be derived from pRS424 (Sikorski and Hieter (1989) *Genetics* 122:19-27). First, primers MO107 (SEQ ID NO:25) and MO197 (SEQ ID NO:26) are used to PCR amplify the lacZ gene from Yep355 (Myers, *et al.*, *Gene* **45**:299-310 (1986)). This lacZ-containing fragment was inserted into the *Bam*HI-*Hind*III sites of pRS416 (Sikorski and Hieter, *Genetics* **122**:19-27 (1989)). This same *lacZ* fragment can be cut out of the resulting vector with *Kpn*I-*Not*I and inserted into the same sites of pRS424 to create pRS424-lacZ. Primers MO1293 (SEQ ID NO:27) and MO1294 (SEQ ID NO:28) are used

to PCR amplify a 2.09 kb fragment of the *lovF* promoter from *A. terreus* genomic DNA. The *lovF* promoter fragment was then cut with *Not*I-*Bgl*II and inserted into *Not*I-*BamH*I linearized pRS424-*lacZ*.

Plasmid MB2114 contains the *lovF*p-*CYC1*p-lacZ reporter gene. It can be derived from pRS424-lacZ (see MB1918 plasmid construction). Primers MO1787 (SEQ ID NO:29) and MO1788 (SEQ ID NO:30) are used to amplify the 264 bp basal *CYC1* element from pRS415 CYC1 (Mumberg, *et al.*, *Gene* **156**:119-122 (1995)). This 264 bp fragment was inserted upstream of the pRS424-lacZ derivative which has been digested with *Spe*I-*Bam*HI. Finally, the *lovF* promoter from MB1918 was PCR amplified with MO1793 (SEQ ID NO:31) and MO1794 (SEQ ID NO:32) and inserted into the *Not*I-*Spe*I sites to create MB2114.

Yeast strains utilized in this study include strains MY2145 and MY2159, which are both derived from the *S. cerevisiae* sigma 1278b strain background; the genotypes are both strains are as follows: *MAT***a** *ura3Δ0 leu2Δ0 his3Δ::hisG trp1Δ0::hisG*. MY2145 and MY2159 contain the *lovF*p-lacZ reporter plasmids MB2114 and MB1918, respectively.

MY2124 transformed with individual *lovE* variant plasmids was mated to *S. cerevisiae* strains MY2154 and MY2159. Diploids were selected on SC-UraTrp media. Multiple diploids from each individual mating were assayed for *lovF*p-*lacZ* expression using 96 well format β-galactosidase assays. For β-galactosidase assays, cells were transferred from transformation plates to 96-well microtiter plates containing 200 μl Z buffer. 12 strains were transferred simultaneously using a 12-channel multi-pipettor to scoop cells from transformation plates. Duplicate samples were prepared for all assays. OD_{600} readings were taken on samples in Z buffer. These values were used to normalize for equal cell number in all assays. After determining OD_{600}, 150 μl of each sample in